

Progress Report
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**CARDIOPULMONARY TOXICITY INDUCED BY AMBIENT PARTICULATE
MATTER
(TRI CITY CONCENTRATED AMBIENT PARTICLE STUDY)**

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EXECUTIVE SUMMARY

The Tri City Concentrated Ambient Particle Study (Tri City CAPS) is designed to investigate the sources and components of fine particulate matter (PM_{2.5}) responsible for adverse health effects, with an emphasis on coal-fired power plant-derived PM. The Project is a multi-site field study to investigate the toxicity of secondary PM_{2.5} derived from coal-fired power plants and other sources, including mobile sources. A portable ambient particle concentrator coupled with a mobile toxicological laboratory are employed to assess the health effects of CAPs in regions dominated by different PM sources. The Project includes three study locations, each to be evaluated during both winter and summer seasons to exploit different meteorological regimes. The first study location is located near the Ambassador Bridge in Detroit, MI, and is heavily influenced by both idling diesel truck traffic and gasoline-fueled commuter traffic. This site also corresponds to the location of an EPRI-funded air pollution epidemiology study. The second site is located in Steubenville, OH, an area dominated by both regional power plant-derived PM as well as local industrial sources. The third site is located in Maurice K. Goddard State Park in Northwest Pennsylvania, an area also heavily influenced by power plant emissions, but lacking urban or industrial influences. The selection of sites and seasons is based on achieving the highest degree of variability in PM composition and contribution from different sources. Spontaneously hypertensive (SH) and normal (Wistar-Kyoto) rats are exposed to CAPs from these locations for 13 consecutive days and assessed for a wide suite of cardiopulmonary endpoints. The rats are implanted with telemeters and evaluated for pulmonary, systemic, and cardiovascular effects. At the same time, comprehensive exposure characterization is carried out to enable linking of adverse health impacts with PM composition. Also, importantly, source apportionment is carried out to enable attribution of toxicological effects to specific PM sources.

This report documents progress made on the Project during the period of January 1, 2006 through March 30, 2006. During this reporting period, laboratory chemical analyses and biological assays were continued on CAPs and PM_{2.5} samples collected during the first round of fieldwork (Detroit, July 16-28; Site 1, Season 1). In particular, trace element analyses in CAPs were completed. Semi-continuous elemental data from the slurry sampler are undergoing QA/QC and will be reported in the next progress report. Processing and data interpretation of cardiac function data collected in Season 1 are continuing.

The second round of fieldwork was successfully completed (Detroit, February 11-23; Site 1, Season 2). For Season 2 fieldwork, the average concentration enrichment factor (CEF) was approximately 30. Mean CAPs concentration during the 13-day exposure period was 368 ± 232 $\mu\text{g}/\text{m}^3$. Black carbon concentrations in CAPs during the exposure period ranged from 1.9 to 14.5 $\mu\text{g}/\text{m}^3$. Additional chemical analyses of both ambient PM_{2.5} and CAPs are in progress.

Toxicological analyses from Season 2 fieldwork suggest little effect of CAPs exposure on airway histology/morphometry or bronchoalveolar lavage parameters. Cardiac function data are currently being analyzed.

During the next reporting period, exposure and toxicological analyses for Site 1 (both seasons) will continue. Site 2, Season 2 fieldwork is scheduled for July 15 – 27, 2006 in Steubenville, OH.

PROJECT PROGRESS

Approach

The monitoring location for the work described in this report is Maybury Elementary School, located at 4410 Porter St. in Southwest Detroit. This site is located ½ mile from the Ambassador Bridge, the busiest border crossing between Canada and the United States with daily traffic volume near this site estimated to be as high as 100,000 vehicles/day. Major contributors to the local traffic congestion are diesel trucks involved in import-export trade, and with materials delivery to and from auto and manufacturing industries in Detroit. Thus, PM at the site is heavily dominated by diesel emission-derived particles. The site is also located 1 block from Interstate 75, the primary southbound route from Detroit to Ohio, thereby contributing gasoline emission-derived PM to exposures conducted at this location.

The Ambassador Bridge area is also being studied as part of the Detroit Exposure and Aerosol Research Study (DEARS), a large EPA-funded exposure assessment study to investigate the importance of different PM sources to residential and personal exposures. DEARS specifically targets the Ambassador Bridge site as being a location heavily impacted by mobile sources. Coupled with DEARS is the EPRI-funded Detroit Cardiovascular Health Study, an epidemiological panel study to be conducted in the same cohort as the exposure study, and at the same time as the proposed Project. Thus, the Project will integrate the toxicology, epidemiology and exposure assessment disciplines for the same site.

The specific approach employed at Site 1, Season 2 was identical to that used in Season 1 fieldwork; additional methodological detail can be found in previous progress reports.

Objectives

The primary objective of the Project is to evaluate the potential for adverse cardiopulmonary effects from ambient exposure to realistic (environmentally relevant) coal-fired power plant and traffic-related PM. Secondary objectives of the study are to (1) provide insight into toxicological mechanisms of PM-induced cardiopulmonary effects, particularly as they relate to susceptible subpopulations; and (2) generate toxicological data to directly correspond to epidemiology and exposure assessment data from concurrent studies being conducted at one of the Project locations, providing a rich dataset of human and animal data exploring the associations between PM sources and components and health.

Results and Discussion

Results are presented for Site 1, Seasons 1 and 2. A subset of results for Season 1 have been previously presented; however, additional exposure analyses are now available.

Site 1, Season 1

Exposure Results

During this reporting period, elemental analysis of the CAPs and ambient PM_{2.5} samples from Season 1 was conducted, which completes the chemical analyses (Tables 1 and 2). Slurry

samples collected from the Semi-Continuous Elements in Aerosol System (SEAS) were also analyzed for trace elements. Quality assurance (QA) and quality control (QC) are being performed on the SEAS sample database, and the data are being compiled for reporting. These data will be available in the next progress report.

Table 1. Chemical composition of CAPs in (concentrations in $\mu\text{g}/\text{m}^3$)

<i>CEFs</i>	<i>Mass^d</i>	<i>OM^b</i>	<i>EC</i>	<i>Sulfate</i>	<i>Nitrate</i>	<i>Ammonium</i>	<i>Urban dust^a</i>	<i>Unidentified</i>	<i>NOTE</i>	
13-day 8-hour exposure										
7/16	15	565.3	108.6	2.6	148.5	29.2	54.6	21.0	200.8	heavy rain event recorded multiple shut-downs of concentrator due to clogging
7/17	65	1448.0	269.3	4.9	378.8	152.1	189.7	43.6	409.6	
7/18	41	1648.7	381.1	10.1	648.4	64.3	252.3	45.4	247.1	
7/19	27	356.2	235.9	7.4	33.3	15.0	20.4	35.1	9.1	
7/20	13	249.3	132.2	15.6	12.8	9.8	10.0	43.4	25.5	
7/21	39	591.9	142.1	3.4	120.7	36.6	57.3	22.7	209.1	
7/22	26	312.5	120.6	4.3	45.1	8.1	22.2	25.7	86.5	
7/23	15	111.0	60.6	2.9	6.0	1.5	6.7	13.8	19.6	rain event in AM recorded
7/24	7	82.4	50.0	5.0	6.1	3.6	5.9	12.9	0.0	
7/25	5	75.2	31.3	1.4	10.8	1.4	8.4	16.2	5.6	
7/26	31	859.0	338.9	6.1	185.7	17.4	91.9	29.7	189.3	
7/27	23	213.1	185.3	8.2	11.0	5.9	12.4	19.5	0.0	
7/28	21	211.5	64.3 ^c	1.6	5.5	11.5	6.4	39.6	82.7	
Average	25	517.2	163.1	5.7	124.1	27.4	56.8	28.3	114.2	

NOTE:

^a Urban dust was calculated from $1.89 \cdot \text{Al} + 1.4 \cdot \text{Ca} + 1.43 \cdot \text{Fe} + 2.14 \cdot \text{Si}$, where Si is estimated by $\text{K}/0.15$

^b Organic mass (OM) was estimated from organic carbon (OC) $\times 1.8$

^c Organic mass (OM) was estimated ambient OM \times CEF (7/28)

^d Average of two filter samples

Table 2: Trace element concentrations in CAPs (concentrations in ng/m³)

	7/16	7/17	7/18	7/19	7/20	7/21	7/22	7/23	7/24	7/25	7/26	7/27	7/28
Mg	244	475	1296	894	1758	477	800	431	189	119	883	279	1383
Al	0	246	941	371	1245	212	875	0	449	308	877	278	755
P	308	179	302	604	426	280	109	146	4	143	267	571	385
S	58163	131986	210868	15925	6033	44243	18950	3790	2998	5473	78273	5074	3372
K	1182	2616	1722	1379	1363	952	1055	651	571	1003	1335	980	1356
Ca	753	753	6414	3502	5903	1302	2388	1488	827	443	3124	924	4712
Ti	17	28	88	51	67	37	55	21	8	8	37	27	71
V	18	16	21	5	7	7	7	4	3	2	13	2	63
Mn	60	94	205	191	411	106	118	39	53	9	190	84	529
Fe	2162	3343	7041	6848	9350	4802	3942	1671	1937	491	3207	2579	8527
Co	0.8	1.8	6.4	2.0	1.9	2.3	1.4	0.6	1.6	0.2	1.5	1.6	3.6
Ni	40	64	211	27	19	70	25	12	43	5	27	49	98
Cu	46	114	116	209	105	120	70	39	24	8	118	61	149
Zn	155	319	769	1629	2419	1192	336	48	35	122	966	272	16737
As	18	56	41	17	11	14	7	5	4	2	17	5	11
Se	70	67	165	20	13	26	15	7	4	3	60	4	12
Rb	2.1	1.6	3.6	1.9	3.9	1.3	1.4	0.7	1.1	0.5	3.6	2.1	4.6
Sr	11.3	58.0	34.3	19.1	38.2	15.5	18.0	8.8	9.3	2.6	17.6	7.2	33.9
Mo	5.5	14.6	62.5	11.2	16.1	18.7	19.0	5.0	4.3	2.6	4.3	16.6	27.3
Cd	2.3	2.3	4.1	5.0	4.0	2.9	1.0	1.0	0.4	0.1	2.4	1.4	7.7
Sb	7.8	39.8	20.9	116.9	74.0	19.4	18.8	5.2	2.9	2.4	9.1	10.7	63.5
La	0.6	1.3	24.4	1.2	22.0	1.1	1.2	0.4	1.3	0.3	6.3	0.3	11.3
Ce	0.8	1.8	16.5	1.9	14.2	1.3	2.0	0.7	1.0	0.3	4.5	0.8	6.4
Sm	0.03	0.04	0.19	0.11	0.18	0.05	0.11	0.03	0.03	0.02	0.13	0.02	0.27
Pb	34	58	336	936	814	129	23	34	10	4	53	16	171

Toxicological Results

Telemetry data (heart rate and heart rate variability) analyses for Season 1 are still underway.

Site 1, Season 2

Exposure Results

Season 2 fieldwork was completed February 11 – 23, 2006. An extensive set of size-segregated aerosol samples was collected in the field during the campaign. Tables 3 and 4 detail the measurements of ambient PM and CAPs; methods were identical to those employed in Season 1 fieldwork.

Table 3. Ambient PM_{2.5} measurements and analyses

Measurement	PM Property	Sampling Medium	Sample Duration (hr)	Analytical Method
TEOM	Mass	-	Continuous	-
SEAS	Trace elements	MQ	Every 30-min	ICP-MS
Filter (PM _{2.5})	Trace elements	Teflon	8	ICP-MS
Filter (PM _{2.5})	Elemental & organic carbon	Quartz	8	TOA

Table 4. CAPs measurements and analyses

Measurement	PM Property	Sampling Medium	Sample Duration (hr)	Analytical Method
TEOM	Mass	-	Continuous	-
Aethalometer	Black carbon	-	Continuous	-
SMPS 3936	Size (0.02-1 μm)	-	Continuous	-
MOI (stage 2)	Size (6 stages) /trace metals	Teflon	8	Gravimetric ICP-MS
Filter	Trace elements	Teflon	8	Gravimetric ICP-MS
Filter	Acid aerosols and ions	Teflon/ denuders	8	Gravimetric pH/IC
Filter	Elemental & organic carbon	Quartz	8	TOA

The concentrations of ambient $\text{PM}_{2.5}$ and CAPs in the animal chamber were used to calculate concentration enrichment factors (CEFs). Ratios of concentrated particle mass concentration to ambient concentration were calculated for each inhalation exposure period to evaluate concentrator performance for each inhalation exposure period (Table 5). The average CEF for the Season 2 fieldwork was approximately 30. As shown, mean CAPs concentration during the 13-day exposure period was $368 \pm 232 \mu\text{g}/\text{m}^3$. Chemical analyses of these filters are in progress.

Table 5. Average ambient $\text{PM}_{2.5}$ and CAPs concentrations, Site 1, Season 2

Date	CEF	CAPs ($\mu\text{g}/\text{m}^3$)	Ambient $\text{PM}_{2.5}$ ($\mu\text{g}/\text{m}^3$)
2/11/06	58	704.0*	12.0*
2/12/06	41	126.4	3.1
2/13/06	41	262.2	6.4
2/14/06	29	564.9	19.2
2/15/06	20	234.0	11.6
2/16/06	29	691.2	23.7
2/17/06	36	57.9	1.6
2/18/06	26	116.1	4.4*
2/19/06	20	202.3	10.1
2/20/06	22	322.8	14.6
2/21/06	20	395.4	19.5
2/22/06	21	703.4	33.6
2/23/06	23	400.4	17.2
Mean	30	368	14

CEF: concentration enrichment factor

* measured by TEOM

During the exposure period, 5-minute average size distribution and number concentrations of submicron particles (< 420 nm) were measured using a Scanning Mobility Particle Sizer (SMPS, TSI Inc) to monitor how ultrafine particle number concentration altered throughout a day. Table 6 shows the number concentration of ultrafine particles (< 100 nm) in the chamber during the exposure period. As shown, the average concentration to which animals were exposed varied on a daily basis. Figure 1 displays the temporal variation in the number concentration of ultrafine particles measured by the SMPS in the chamber during the exposure period on February 22nd. Both the mean and maximum concentrations of ultrafine particles on this day were highest of all the days in the 13-day exposure period. Interestingly, the dominant wind direction during the exposure period on this day was south-to-southwest, where the densest industrial activity is located. Furthermore, black carbon concentrations also were highest on this day (Table 7). Therefore, the spikes shown in the midday in Figure 1 are likely to have resulted from the southwest industrial community. More continuous data will be compiled for the next progress report.

Table 6. Number concentration of ultrafine particles, Site 1, Season 2

Date	n	Mean	Median	Min	Max	SD
2/11/06	96	1.3E+04	1.6E+04	4.3E+02	2.5E+04	7.4E+03
2/12/06	95	1.0E+04	9.8E+03	5.8E+03	2.0E+04	2.7E+03
2/13/06	96	9.2E+03	8.7E+03	4.2E+03	2.1E+04	3.1E+03
2/14/06	90	1.2E+04	1.2E+04	5.9E+03	2.3E+04	3.6E+03
2/15/06	95	1.4E+04	1.1E+04	4.7E+03	3.0E+04	7.6E+03
2/16/06	96	1.2E+04	1.2E+04	6.8E+03	2.6E+04	3.2E+03
2/17/06	96	3.9E+03	3.8E+03	1.6E+03	6.5E+03	1.2E+03
2/18/06	97	4.9E+03	4.2E+03	9.1E+02	9.0E+03	2.2E+03
2/19/06	97	1.4E+04	1.3E+04	3.4E+03	2.8E+04	3.8E+03
2/20/06	95	1.6E+04	1.4E+04	7.7E+02	4.0E+04	9.6E+03
2/21/06	96	8.0E+03	6.1E+03	2.1E+03	2.3E+04	5.2E+03
2/22/06	95	2.0E+04	2.0E+04	5.9E+03	4.5E+04	1.1E+04
2/23/06	97	1.5E+04	1.1E+04	1.5E+03	4.0E+04	1.1E+04
Average		1.2E+04	1.1E+04	4.3E+02	4.5E+04	3.4E+03

Concentration in cm⁻³; n: sample size; SD: standard deviation

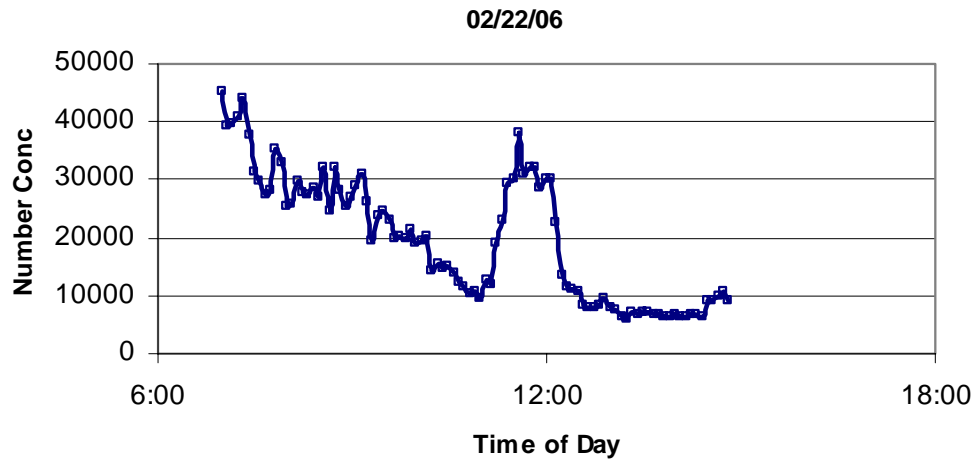


Figure 1. Temporal variations in ultrafine number concentration as measured by the SMPS on February 22, 2006.

Table 7. Black carbon concentrations in the exposure chamber as measured by the aethalometer, Site 1, Season 2

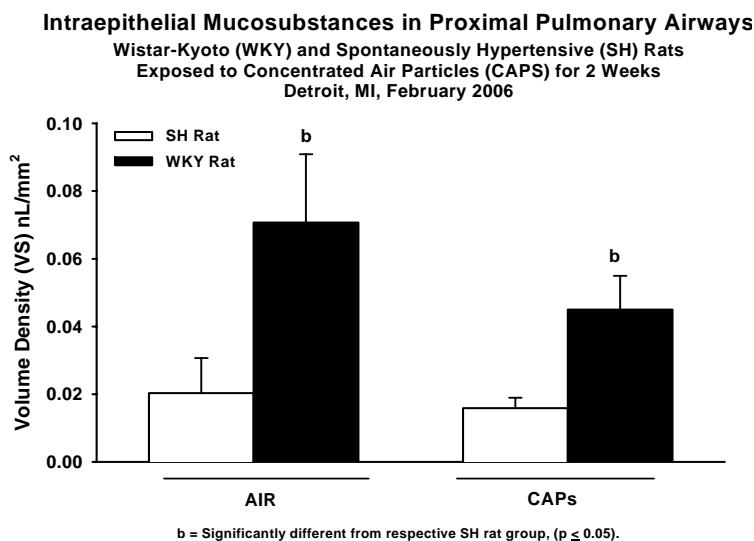
	Average	STD	Min	Max
2/11/06	9108	3459	4527	18749
2/12/06	4270	2276	2525	14116
2/13/06	5870	1776	3030	9948
2/14/06	10425	1886	6126	14416
2/15/06	9827	1159	7656	12536
2/16/06	12419	1555	9319	14845
2/17/06	1931	820	862	6178
2/18/06	2085	416	1028	3494
2/19/06	4372	1862	2868	10886
2/20/06	7385	2006	5475	12380
2/21/06	7134	1880	4259	11410
2/22/06	14523	4152	7018	23369
2/23/06	7826	3614	3402	17711

Concentrations in ng/m^3 ; STD: standard deviation

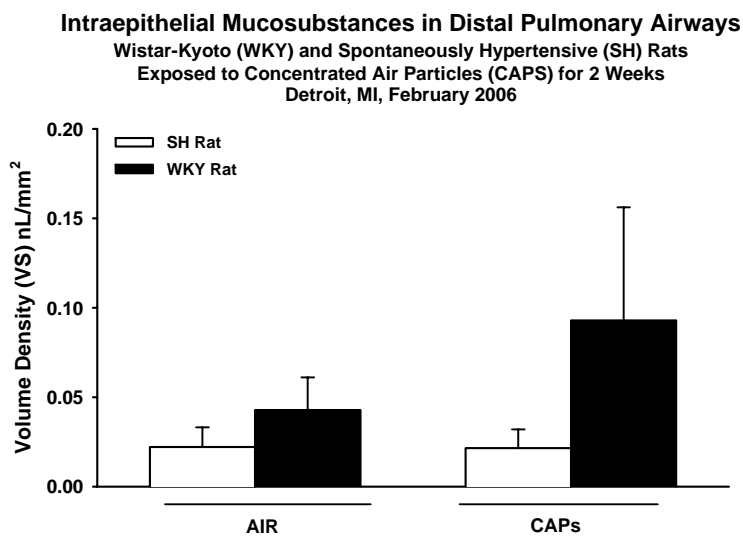
Toxicological Results

Airway histology and morphometry: Fixed nasal and pulmonary tissues were processed for routine histological examination and morphometry of intraepithelial mucosubstances (IM), as described in the last progress report. Air-exposed WKY rats had significantly more IM in proximal pulmonary airways compared to SH rats, and CAPs exposure had no effect on IM in either strain of rat (Figure 2a). No significant strain or exposure differences were observed in distal pulmonary airways (Figure 2b). By comparison, in nasal respiratory epithelium, WKY rats had significantly less IM than SH rats (Figure 2c). Similar to the response to CAPs exposure in pulmonary airways, IM was unaffected in the nose.

(a)



(b)



(c)

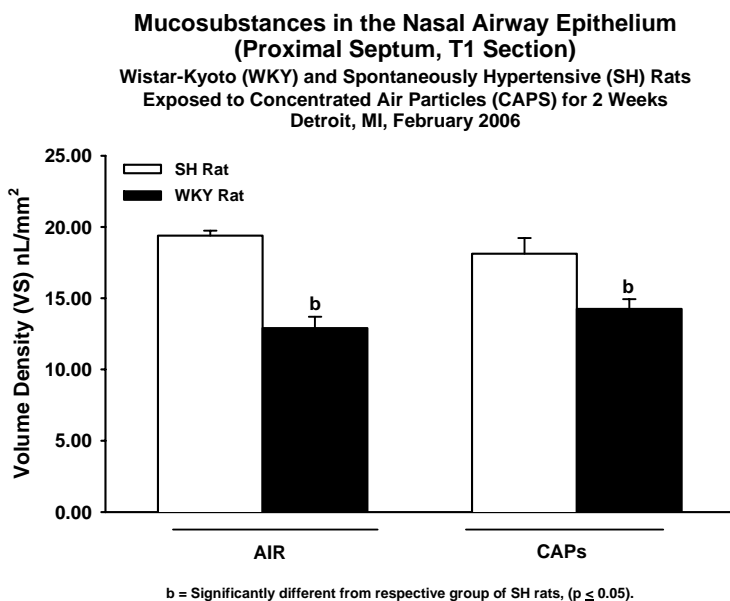


Figure 2. Intraepithelial mucosubstances in air- and CAPs-exposed rats, Site 1, Season 2. (a) proximal pulmonary airways; (b) distal pulmonary airways; (c) nasal airway epithelium.

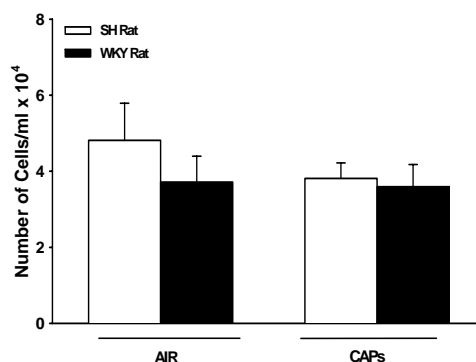
These results are different from what we documented in July 2005 in Detroit, where CAPs exposure caused statistically significant increases in IM in pulmonary airways of WKY rats (see last progress report). Airways in SH rats were unaffected by CAPs exposure in this study. We interpret the difference in the changes in IM as being driven by physicochemical characteristics (i.e., composition) of CAPs during these two 13-day exposures. It should be noted that amounts of IM in all experimental groups was relatively low, and does not suggest significant airway lesions or notable pathology. For comparison, we routinely see IM levels of 0.5-3.5 nl/mm² in pulmonary airways of laboratory rodents with experimental allergic airway disease or chronic obstructive pulmonary disease.

Analyses of bronchoalveolar lavage (BAL) fluid: Analysis of lavage cellularity indicated some minor strain differences, but no CAPs-induced effects (Figure 3). WKY rats displayed a trend toward less overall cellularity in BAL fluid, due to fewer numbers of both neutrophils and macrophages compared to SH rats. CAPs exposure did not significantly affect BAL cellularity in February. Plans to determine TNF- α in BAL fluid have been postponed to potentially run these samples using a cytometric bead array assay, which can determine as many as 6 cytokines in a single sample. Other inflammatory cytokines such as IL-6 and IL-10 may be added to the analysis.

(a)

Total Number of Cells in Bronchoalveolar Lavage Fluid (BALF)

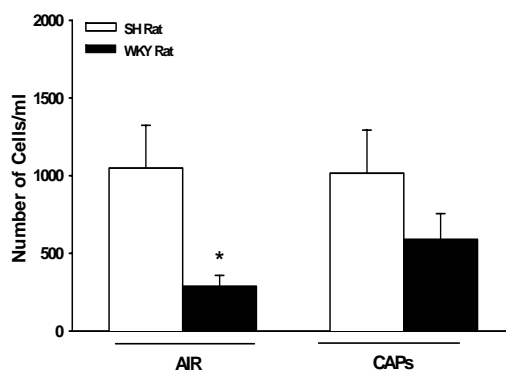
Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SH) Rats
Exposed to Concentrated Air Particles (CAPS) for 2 Weeks
Detroit, MI, February 2006



(b)

Neutrophils in Bronchoalveolar Lavage Fluid (BALF)

Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SH) Rats
Exposed to Concentrated Air Particles (CAPS) for 2 Weeks
Detroit, MI, February 2006



* = Significantly different from SH receiving Air, ($p \leq 0.05$).

(c)

Macrophages in Bronchoalveolar Lavage Fluid (BALF)

Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SH) Rats
Exposed to Concentrated Air Particles (CAPS) for 2 Weeks
Detroit, MI, February 2006

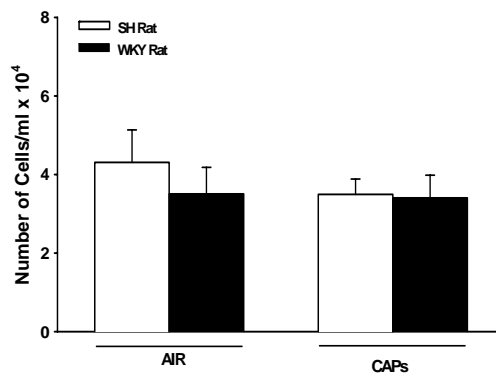


Figure 3. BAL cellularity, Site 1, Season 2. (a) total BAL cells; (b) neutrophils; (c) macrophages.

Electrocardiograms and telemetry: Continuous data recordings of heart rate, temperature, and ECG waveforms were collected during both the exposure period (i.e., 7:00AM – 3:00PM), and during non-exposure hours of 4:00PM – 6:00AM. Analyses of these time periods will allow us to track real-time, immediate responses during particle inhalation, and to determine any lag effects in the near-term, or activity-dependent responses during evening hours when rats are awake. Interbeat-intervals for all recording periods have been extracted from the raw data acquisition files and are ready to be processed for determination of HRV variables of SDNN (using SAS: Statistical Analysis Software) and rMSSD (MS Excel). These analyses are presently incomplete. ECG waveform analysis is also planned and will be performed with a new version of ECG Analysis software from Data Sciences International, and will be available in June.

Conclusions

We have successfully completed the second field sampling campaign for the Project. Remaining exposure and toxicological analyses are underway and expanded results will be reported in the next progress report (July 31, 2006). Fieldwork for Site 2, Season 1 is scheduled for July 15 – 27, 2006 (Steubenville, OH).

COST STATUS

The table below summarizes the budget and expenditures to date. These costs include subcontractor costs (University of Michigan, Michigan State University).

Total federal funds authorized for this funding period	\$831,182
Total outlays	\$70,972
Recipient share of outlays	\$19,407
Federal share of outlays	\$51,565

SCHEDULE STATUS

The project is on schedule. We anticipate no problems in meeting the next milestone, Completion of Field Experiments at Site 1, by 6/30/06. Overall progress on the Project tasks is shown in the Table below.

Technical Progress -- 9 months

Task #	Description	Planned % completed	Actual % completed
1	Field Experiments at Site 1, Season 1	100%	95%
2	Field Experiments at Site 1, Season 2	60%	60%
3	Data Analysis for Site 1	57%	30%
4	Field Experiments at Site 2, Season 1	0%	0%
5	Field Experiments at Site 2, Season 2	0%	0%
6	Data Analysis for Site 2	0%	0%
7	Field Experiments at Site 3, Season 1	0%	0%
8	Field Experiments at Site 3, Season 2	0%	0%
9	Data Analysis for Site 3	0%	0%
10	Integrated Data Analysis for All Sites	0%	0%
11	Project management and reporting	19%	19%

SUMMARY OF SIGNIFICANT ACCOMPLISHMENTS

We successfully completed fieldwork at Site 1, Season 2. Data processing and analysis for sampling and toxicological assessment in both seasons are continuing, and fieldwork for the next study location (Steubenville) has been scheduled.

ACTUAL/ANTICIPATED PROBLEMS

No problems were encountered. We do not expect any problems for the next round of fieldwork, scheduled for July 15 - 27, 2006.

TECHNOLOGY TRANSFER ACTIVITIES

Dr. Annette Rohr presented a paper entitled "Health Effects of Coal Combustion Emissions", which included results from the subject Project, at the Electric Utilities Environmental Conference, Tucson, AZ, January 23, 2006.